

Murine and human Langerhans cells express a functional histamine H₄ receptor: modulation of cell migration and function

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Abstract

Background: Histamine is an important mediator of allergic reactions, and recent studies indicated that the function of different types of antigen presenting cells (APC) can be modulated by histamine, in particular via the newly described histamine H₄ receptor (H₄R). Therefore, we investigated possible interactions of histamine via the H₄R on Langerhans cells (LC), which represent the professional APC in the skin and therefore have an important role in the initiation and maintenance of allergic skin diseases.

Methods: The expression of the H₄R was evaluated by real-time PCR, flow cytometry and immunofluorescence staining. The function of the H₄R was determined by intracellular flow cytometric measurement of chemokine production and LC migration assays.

Results: Here, we show H₄R expression on *in vitro* generated monocyte-derived LC (mRNA and protein) and on primary LC from murine and human skin samples (protein). The immunofluorescence staining in murine and human skin samples clearly proved that LC express the H₄R *in situ*. Stimulation with histamine or a H₄R agonist downregulated the chemokine (C-C motif) ligand 2 (CCL2) in human monocyte-derived LC and primary LC. Prestimulation with a selective H₄R antagonist abolished this effect. Moreover, migration of LC from the epidermis was increased after H₄R agonist stimulation in *ex vivo* migration assays using human epidermis and murine *in vivo* assays.

Conclusion: Our findings show that LC express a functional H₄R and point towards a possible pathogenic relevance of the H₄R in inflammatory and allergic diseases.

Langerhans cells (LC) are the sentinels of the immune system in the skin. In case of antigen or allergen

encounter, LC migrate from the epidermis to the local lymph nodes to initiate a T cell response. Because of this pivotal role of LC in the establishment of cutaneous allergic immune responses, we were interested in studying effects of the allergic mediator histamine on these cells. During inflammatory and allergic reactions, high amounts of histamine are released in the skin,

Abbreviations

APC, antigen presenting cells; DC, dendritic cells; H₄R, histamine H₄ receptor; LC, Langerhans cells; MoDC, monocyte-derived dendritic cells; MoLC, monocyte-derived langerhans cells.

which acts via four distinct G-protein-coupled histamine receptor subtypes (H₁R-H₄R) (1). These receptors are expressed on many different cell types and therefore play diverse roles: the H₁R is widely distributed and closely related to the development of allergic conditions, because of its influence on vascular permeability, smooth muscle and endothelial cell contraction. The H₂R is mainly involved in the secretion of gastric acid (2) but is also present on other cell types such as smooth muscle cells and certain leucocytes. The H₃R is primarily expressed in the central nervous system and was identified to be an autoreceptor that negatively regulates the release of histamine and other neurotransmitters (3). The recently discovered H₄R (4, 5) is present on many cell types involved in inflammatory and allergic diseases, such as T cells, dendritic cells, mast cells and eosinophils, and immunomodulatory functions in the murine as well as the human system have already been identified. H₄R stimulation was shown to induce chemotaxis of mast cells (6), monocyte-derived dendritic cells (MoDC) (7) and eosinophils (8). In monocytes, MoDC and monocyte-derived inflammatory epidermal dendritic cells, H₄R-dependent suppression of IL-12 and CCL2 production were described (7, 9, 10).

These findings implicate an important role of histamine in modulating the function of antigen presenting cells (APC). Therefore, histamine might also influence LC, whose interaction with histamine has not been extensively investigated until now. Previous studies showed that LC do not express the H₁R and the H₂R on mRNA level (11). Thus, it is possible that histamine might modulate LC function via the H₄R during allergic skin reactions. We intended to study the expression and function of the H₄R on human LC generated *in vitro* from monocytes and on primary LC isolated from human skin (*ex vivo* studies). Moreover, we investigated the H₄R on murine LC to get more insight into the comparability of H₄R-mediated responses in human and mice.

Materials and methods

Human skin samples

Samples from healthy skin were obtained during plastic surgery or safety margin operations of skin tumours from anonymous donors directly after surgery. The investigation of the role of histamine receptors in allergic skin inflammation was approved by the local ethics committee of the Hannover Medical School (Vote Nr. 4253) and was conducted according to the Declaration of Helsinki Principles.

Mice

BALB/c and NMRI mice were obtained from Charles River (Sulzfeld, Germany) at the age of 6–8 weeks. The H₄R-deficient (H₄R^{-/-}) mouse was kindly provided by Robin Thurmond (Johnson & Johnson, La Jolla, CA, USA). Mice were bred in the animal facility of the Veterinary University Hannover. All animals were healthy and were housed in groups of six mice per cage at 22°C with a 12-h light/dark cycle. Water and standard diet (Altromin, Lage/Lippe, Germany) were available *ad libitum*. The animal experiments have been approved by Bezirksregierung Hannover, Germany (AZ G33-42502-06/1126).

Preparation of epidermal single cell suspensions

Single cell suspensions were prepared as described previously (12). The cells were cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 100 mg/ml penicillin/streptomycin, 12 mM Hepes and 5% v/v foetal calf serum (PAN-Biotech; all other media components from Biochrom, Berlin, Germany) at 37°C in a humidified atmosphere containing 5% CO₂.

Single cell suspensions from mouse ear skin were prepared as follows: Mice were killed, and ears were cut and split into dorsal and ventral halves. The epidermis was separated from the dermis after incubation of the ear halves in 0.5 M ammonium thiocyanate (Riedel de Haën, Hannover, Germany) at 37°C for 10 min. After washing of the epidermis in phosphate buffered saline (PBS), single cell suspensions were prepared analogously to human samples.

Preparation of spleen cell suspensions

Mice were killed; the spleen was taken out and minced. The spleen homogenate was filtered through a sterile gauze (40 µm), and the cells were washed in PBS. Red blood cells were lysed using RBC lysis buffer (eBioscience, San Diego, CA, USA). After another washing step, the cells were cultured in Iscove medium supplemented with 2 mM L-glutamine, 100 mg/ml penicillin/streptomycin, 0.05 mg/ml gentamycin, 1× nonessential amino acids and 10% v/v foetal calf serum (PAN-Biotech, all other components from Biochrom AG) at 37°C in a humidified atmosphere at 5% CO₂.

Generation of monocyte-derived LC

Buffy coats from anonymous healthy donors were obtained from the local blood bank. Peripheral blood

mononuclear cells were separated from buffy coats by density centrifugation on lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway). Adherent cells were obtained by plastic adherence: 5×10^7 peripheral blood mononuclear cells were plated in 80-cm² culture flasks (NuclonTMD Nunc GmbH & Co. AG, Wiesbaden, Germany) in supplemented RPMI 1640 medium for 1 h (37°C, 5% CO₂, humidified atmosphere). The non-adherent cells were removed by vigorous washing with PBS. The adherent cells (enriched monocytes, purity at least 85%) were further cultured in supplemented RPMI 1640 and for the generation of MoLC, 10 ng/ml IL-4 (R&D Systems, Wiesbaden, Germany), 10 ng/ml granulocyte-monocyte colony-stimulating factor (Berlex Pharmaceutical Company, Montville, NJ, USA), 10 ng/ml TGF- β (Promokine, Heidelberg, Germany) and 10 mM β -mercaptoethanol (Sigma-Aldrich, Taufkirchen, Germany) were added. The medium was changed every 2–3 days, and the cells were harvested on day 8. The cells were characterized as MoLC by staining for the following surface markers: CD1a (BD Biosciences, Bedford, MA, USA), CD14, CD36, CD40, CD54, CD80, CD83, CD86 (all from Beckman Coulter, Krefeld, Germany), CD206 (BD Biosciences), CD207 (Beckman Coulter) and HLA-DR (BD Biosciences) or the respective isotypes (Beckman Coulter and Sigma-Aldrich).

Flow cytometric analysis of H₄R expression

For H₄R staining, the cells isolated from human epidermis were either used directly after single cell preparation or after overnight culture. The cells were washed twice in PBS and then incubated for 20 min in PBS containing human immunoglobulin G (IgG) (Fc γ R-blocking buffer). To distinguish LC from the other cells present in the suspension, they were stained with anti-CD207-PE (Langerin; Beckman Coulter) or mIgG1-PE as isotype control (Sigma, Taufkirchen, Germany). Subsequently, cells were fixed and permeabilized (Fixation/Permeabilization Kit, eBioscience). Intracellular staining was performed with H₄R antibody recognizing amino acids 194–303 (SantaCruz Biotechnology, Santa Cruz, CA, USA) or polyclonal rabbit isotype control (R&D Systems), followed by labelling with goat anti-rabbit-FITC (Beckman Coulter). CD207 and H₄R positivity of the cells was assessed by flow cytometry (FACS Calibur; Becton Dickinson, Heidelberg, Germany).

For H₄R evaluation on murine LC, the cross reactivity of the human anti-H₄R antibody for the murine system was confirmed by western blot experiments with murine spleen cells (data not shown). The analy-

sis by flow cytometry was performed analogously to human cells, using anti-CD207-Alexa488 clone 929F3 (AbCys, Paris, France) or ratIgG2a-FITC (Beckman Coulter) as isotype control for surface staining. H₄R antibody (SantaCruz Biotechnology) or isotype control (R&D Systems), goat anti-rabbit-biotin (Jackson Immuno Research, Suffolk, UK) and streptavidin-PerCP (BD Pharmingen) were used for the intracellular detection of H₄R. In mouse spleen cell suspensions, intracellular staining was performed with H₄R antibody (SantaCruz Biotechnology) or polyclonal rabbit isotype control (R&D Systems), followed by labelling with goat anti-rabbit-FITC (Beckman Coulter).

Immunofluorescence staining of the H₄R on human LC

Frozen sections (5 μ m) from skin biopsies were transferred to SuperFrost Plus microscope slides (Menzel-Gläser, Braunschweig, Germany). After drying, the sections were fixed in ice-cold 99% ethanol, then the sections were incubated in horse serum (Vectastain ABC kit, alkaline phosphate mouse IgG; Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature (RT). Following this, the slides were stained with anti-CD207 (Beckman Coulter) or mIgG1 (R&D Systems) overnight at 4°C, secondary antibody (biotinylated horse anti-mouse antibody, Vectastain ABC kit, alkaline phosphatase mouse IgG; Vector Laboratories) for 1 h at RT and avidin-fluorescein (Vector Laboratories) for 5 min at RT. After washing, the sections were incubated in goat serum for 1 h at RT and then stained with anti-H₄R (SantaCruz Biotechnology) for 3–4 h and goat anti-rabbit-APC (Beckman Coulter) for 30 min. After a washing step, the stained sections were covered with mounting medium (Vectashield; Vector Laboratories) and coverslips. Fluorescence images were obtained with a Zeiss Axiolab microscope with AxioCam MRm, and images were acquired with the program AxioVS40 (version 4.6.1.0) (Carl Zeiss MicroImaging GmbH, Göttingen, Germany).

Immunofluorescence staining of the H₄R on murine LC

Epidermal sheets were prepared and evaluated as described previously (13). In short, ear skin was floated on 0.5 M ammonium thiocyanate for 10 min at 37°C. LC were detected with biotinylated monoclonal rat anti-mouse MHC-II (Becton Dickinson) and streptavidin-carbocyanin2 (Dianova, Hamburg, Germany). H₄R was stained with anti-H₄R (SantaCruz Biotechnology) and goat anti-rabbit-carbocyanin3 (Dianova).

Intracellular staining of CCL2

Single cell suspensions or *in vitro* differentiated MoLC were stimulated with 10 μ M histamine (Alk-Scherax, Wedel, Germany) or clobenpropit (Sigma) for 48 h before intracellular staining of CCL2 was performed. For blocking experiments, cells were incubated with JNJ7777120 [synthesized as described previously (14)] 30 min before stimulation. The cells of the skin suspension were washed in PBS, and after incubation with Fc γ R-blocking buffer, the surface was stained with anti-CD207-PE (Beckman Coulter) or mIgG1-PE (Sigma). After fixation and permeabilization (Fixation/Permeabilization Kit, eBioscience), intracellular staining was performed with anti-CCL2-APC (R&D Systems) or mIgG2b-APC (R&D Systems). CD207 and CCL2 positivity of the cells was quantified by flow cytometry. *In vitro* differentiated MoLC were permeabilized and stained for intracellular CCL2.

Ex vivo human LC migration assay

Epidermis samples were either taken from blisters of patients suffering from bullous pemphigoid or the epidermis of healthy skin was separated from the dermis after 4°C overnight incubation in 2.4 U dispase II (Roche, Mannheim, Germany). The epidermis samples were washed two times, cut into pieces of approximately equal size and placed in 2 ml of supplemented RPMI 1640 in a 6-well plate. Stimulation was performed with 10 μ M histamine or 4-methylhistamine (Tocris Bioscience, Bristol, UK). For blocking experiments, cells were incubated with JNJ7777120 30 min before stimulation. The next day, the skin was transferred into a plate containing new medium with fresh stimuli to give another impulse for migration. After another 24 h of incubation, the epidermis was dried to allow measurement of the weight of the individual skin pieces. The medium was pooled, and the amount of migrated cells was counted using a haemocytometer. To evaluate the amount of cells that migrated from the epidermis to the culture medium, the cell number per mg of skin was calculated. After assay completion, the cells were washed twice in PBS and used for further analysis by real-time PCR and flow cytometry to prove the identity of LC.

mRNA isolation, reverse transcription and quantitative RT-PCR

Nonstimulated murine spleen cells and human MoLC, keratinocytes, T cells, monocytes, macrophages, eosinophils and the cells remaining after the migration

assay were washed in PBS and lysed for RNA isolation using Mini RNA Isolation II Kit (Zymo Research, Orange, USA), and reverse transcription was carried out using the First-Strand cDNA Synthesis Kit (MBI Fermentas, St. Leon-Rot, Germany). Real-time quantitative PCR was performed on a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) using SYBR Green with Quantitect primer assays for human glyceraldehyde-3-phosphate dehydrogenase GAPDH (QT01192646), human CD207 (QT00045689), human H₄R (QT00032326), murine glyceraldehyde-3-phosphate dehydrogenase GAPDH (QT01658692) and murine H₄R (QT00135884) according to manufacturer's instructions (Qiagen, Hilden, Germany). The following settings were used: an initial activation step of 15 min at 95°C with ramp 20°C/s was followed by three-step cycling (45 cycles): denaturation 15 s, 94°C; annealing 20 s, 55°C; extension 20 s, 72°C (all three with ramp 2°C/s). Melting curve analysis was performed from 60 to 90°C with ramp 20°C/s. The amount of target genes relative to the reference GAPDH was quantified using the Relative Quantification Software (Roche Molecular Biochemicals). To visualize the amplification products after completion of the PCR run, agarose gel electrophoresis was performed with 2% agarose (Roth, Karlsruhe, Germany) in 1 $\times$ Tris-Borate-EDTA buffer (Roth). Samples were diluted in 6 $\times$ loading dye (Fermentas) and Gene Ruler 100 bp plus DNA

In vivo murine LC migration assay

BALB/c mice were narcotized by intraperitoneal injection with ketamine/xylazine (Alvetra, Neumünster and CP-pharma, Burgdorf, Germany). Twenty microlitres of 10 μ M 4-methylhistamine was injected intradermally into the left ear and 20 μ l PBS into the right ear. After 5 h, the procedure was repeated. 24 h after the second administration, the mice were killed, the ears were separated into dorsal and ventral halves, and staining of MHC-II was performed as described previously. Analysis was carried out using the Axiovision (Carl Zeiss, Göttingen, Germany) image analysis system. The density of LC was analysed at 20 $\times$ magnification on a calibrated grid. Sixteen randomly chosen areas were counted per ear, and the number of LC/mm² of epidermis was calculated.

Statistical analysis

For statistical evaluation, a normality test was performed. Because all data sets were normally distributed, the paired *t*-test was used for statistical

calculations. A *P*-value below 0.05 was regarded as significant. *P* < 0.05 is depicted with * and *P* < 0.005 with **. The program GRAPHPAD PRISM version 3.02 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis.

Results

Human and murine LC express the H₄R

To evaluate the expression of the H₄R on LC, we initially generated LC *in vitro* from human monocytes (MoLC) and performed real-time PCR experiments and flow cytometric measurement. MoLC express the H₄R on mRNA as well as on protein level as shown in Fig. 1. Because *in vitro* generated MoLC do not completely resemble naturally occurring LC, we additionally studied *ex vivo* LC obtained in epidermal single cells suspensions. H₄R expression on *ex vivo* human LC was shown by double colour staining of langerin (CD207) and H₄R. Langerin-positive cells expressed the H₄R as shown by flow cytometric measurements of epidermal cell suspensions (Fig. 2A,B) and by immu-

nofluorescence staining of human healthy skin (Fig. 2 C–F).

In skin sections of BALB/c mice, we could show the expression of the H₄R on LC by immunofluorescence staining, while the staining with the same antibody in the skin of H₄R-deficient mice lead to no signal detection (Fig. 3A). To demonstrate specificity of our H₄R detection methods, we performed flow cytometric measurements and real-time PCR assays of the H₄R on spleen cell suspensions from wild-type mice and H₄R^{−/−} mice (Fig. 3B,C, respectively). Flow cytometric analysis of single cell suspensions isolated from murine ear skin showed that murine LC, identified by positive staining for langerin, express the H₄R (Fig. 3D). Because it was described that the sensitivity to histamine varies between two strains – BALB/c and NMRI – as shown by different scratching behaviour in response to histamine (15), we investigated whether the level of H₄R expression on LC can be linked to this response. We found that H₄R expression on LC did not differ between the two investigated strains, the histamine-sensitive NMRI and nonhistamine-sensitive BALB/c mice, as summarized in Fig. 3E.

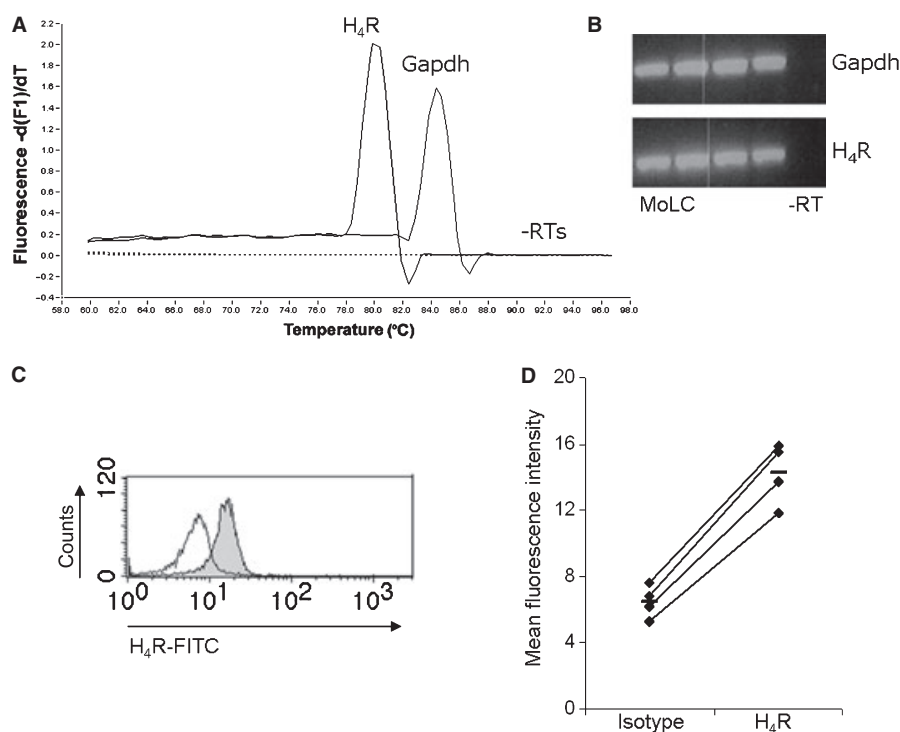


Figure 1 The H₄R is expressed on human monocyte-derived Langerhans cell on the mRNA and protein level. A representative real-time PCR experiment with specific melting peaks for the H₄R and Gapdh and a reaction without reverse transcriptase as a nega-

tive control (A). A representative agarose gel with the PCR products (B). One representative flow cytometric experiment showing H₄R staining (grey) when compared to isotype staining (white) (C) out of four which are summarized in (D).

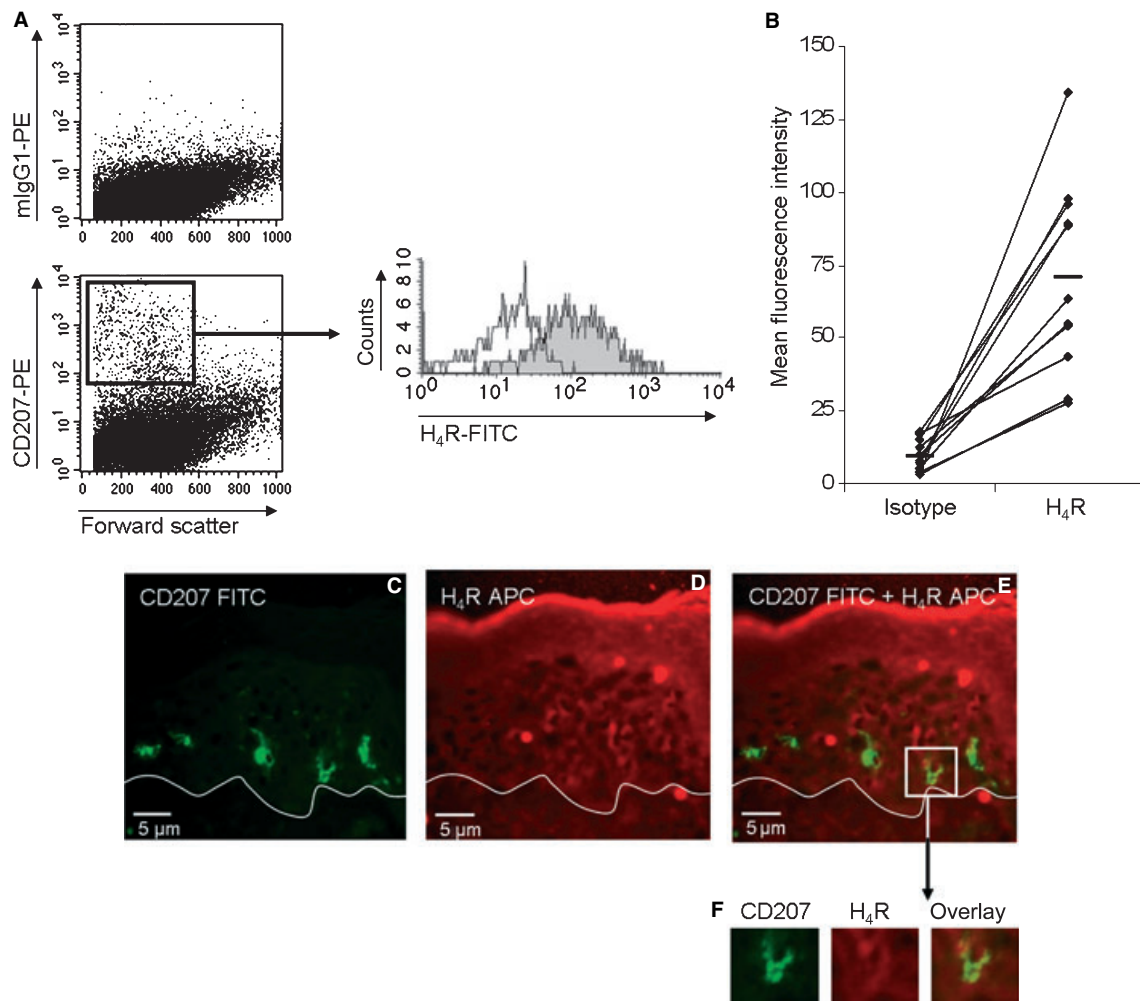


Figure 2 The H₄R is expressed on human naturally occurring Langerhans cell (LC). One to two percentage of total cells in epidermal cell suspensions were CD207-positive LC as assessed by flow cytometry (A). The histogram shows H₄R staining (grey) and isotype staining (white) for the CD207-positive cells of one representative experiment out of 11, which are summarized in

(B). Immunofluorescence staining in the skin with anti-CD207 (langerin) (C) and anti-H₄R (D) shows colocalization of the H₄R on CD207-positive LC (E), enlarged in (F). One representative experiment of 4 is shown. Location of the basement membrane is indicated by the white line. Of note, keratinocytes express also H₄R.

Inhibition of CCL2 production in human LC by H₄R activation

After showing that the H₄R is expressed on human and murine LC, we were interested whether receptor stimulation influences the function of LC. Because we have already described previously that H₄R stimulation decreases the level of CCL2 in human monocytes (9) and IDEC (10), we measured CCL2 levels in human LC. After 48 h of stimulation of human epidermal single cell suspensions with 10 μM histamine or the H₄R agonist clobenpropit, we observed a down-regulation of CCL2 levels in langerin-positive cells (Fig. 4A). The decrease in chemokine production was

blocked by preincubation with the H₄R selective antagonist JNJ7777120 (Fig. 4B), proving that the effect is specific for H₄R activation. Also, in *in vitro* generated MoLC, we could detect lower levels of CCL2 after the activation of H₄R (Fig. 4C).

Histamine-induced *ex vivo* migration of human LC

We investigated the influence of histamine on the migration of LC from the human epidermis, because it has recently been shown that H₄R stimulation induces migration of murine bone-marrow-derived DC and skin DC (15) and human monocyte-derived DC (7). In a LC migration assay using epidermis derived from patients

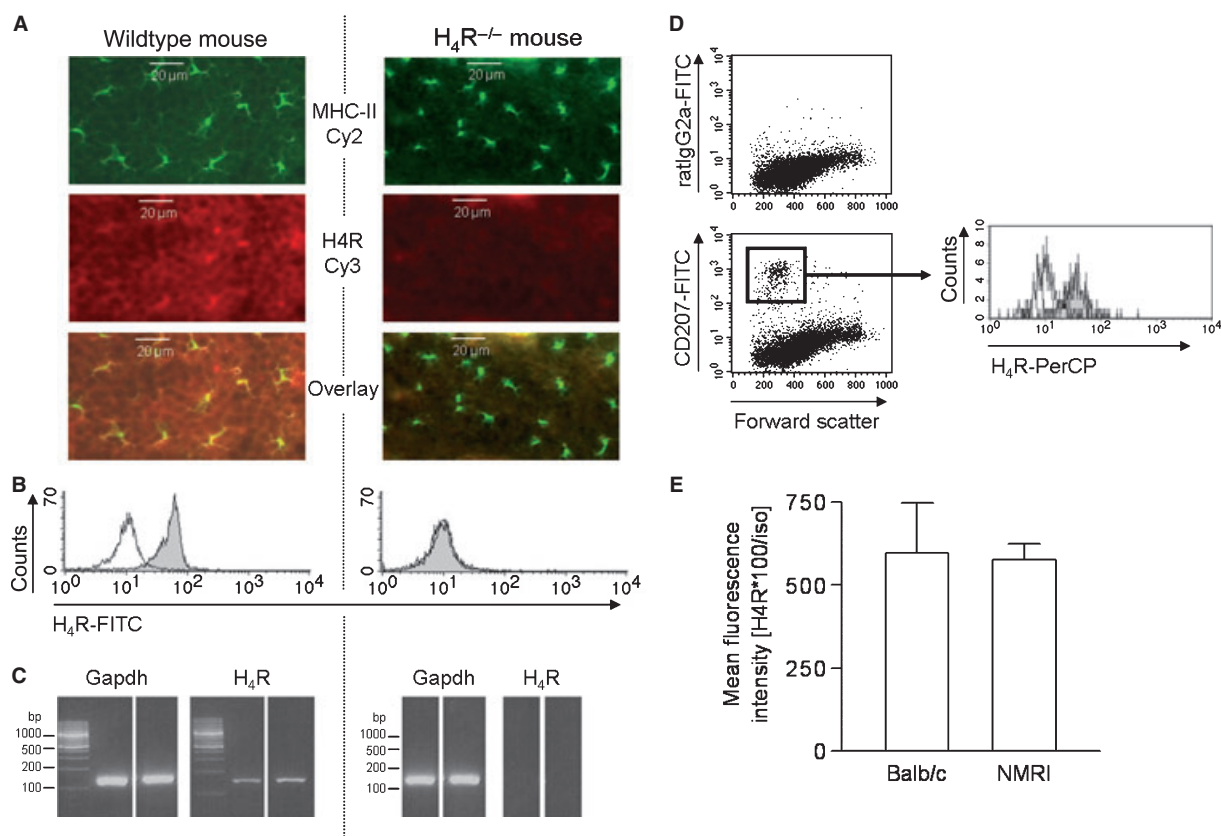


Figure 3 The H₄R is expressed on murine Langerhans cell (LC) and murine spleen cells. The H₄R is expressed on MHC-II-positive LC in the wild-type BALB/c mouse (left panel) and absent on LC in the $H_4R^{-/-}$ mouse (right panel) (A, one representative experiment of $n = 6$ and $n = 2$, respectively). Confirmation of H₄R staining on spleen cells from wild-type BALB/c and $H_4R^{-/-}$ mice (B, H₄R: grey; isotype: white; one experiment out of three). Presence of H₄R

mRNA in the wild-type and absence of H₄R mRNA in $H_4R^{-/-}$ mice as shown in the agarose gel electrophoresis of RT-PCR products (C, one experiment out of three is shown). One to two percentage of total cells from mouse epidermal cell suspensions were CD207-positive LC, expressing the H₄R (grey) when compared to isotype control (white) (E). LC from BALB/c and NMRI mice do not differ in H₄R expression (F, $n = 4$ and $n = 3$, respectively).

with bullous pemphigoid, significantly increased LC migration was induced by histamine and the H₄R agonist 4-methylhistamine (Fig. 5A). Preincubation with the H₄R antagonist JNJ777120 inhibited the histamine-induced migration of LC, proving that the effect is specific for H₄R (Fig. 5A). Disease-specific effects seem to play no role, because the H₄R agonist 4-methylhistamine also induced migration of LC from the epidermis derived from healthy donors (Fig. 5B). The migrated cells represent LC, as shown by real-time PCR (Fig. 5C) and flow cytometry (Fig. 5D).

Histamine-induced *in vivo* migration of murine LC

In a previous study, migration of skin DC induced by H₄R stimulation was shown by *ex vivo* migration assays (15). To show that this functions as well *in vivo*, we performed *in vivo* LC migration experiments in the mouse.

After intradermal injection of 4-methylhistamine into the mouse ear, the density of LC significantly decreased when compared to the ear of the contralateral site which was injected with PBS (Fig. 6A,B). These results showed that H₄R stimulation can induce the migration of LC also *in vivo* and results in a lower number of LC present in the epidermis.

Discussion

In this study, we investigated a possible effect of histamine on Langerhans cells (LC), because they are likely to come into contact with histamine at sites of allergic skin diseases, and histamine has been shown to modulate the function of other dendritic cell subtypes. We focused on the H₄R, because a previous study suggested the absence of the H₁R and the H₂R on LC (11), and the H₃R is not likely to be expressed on LC,

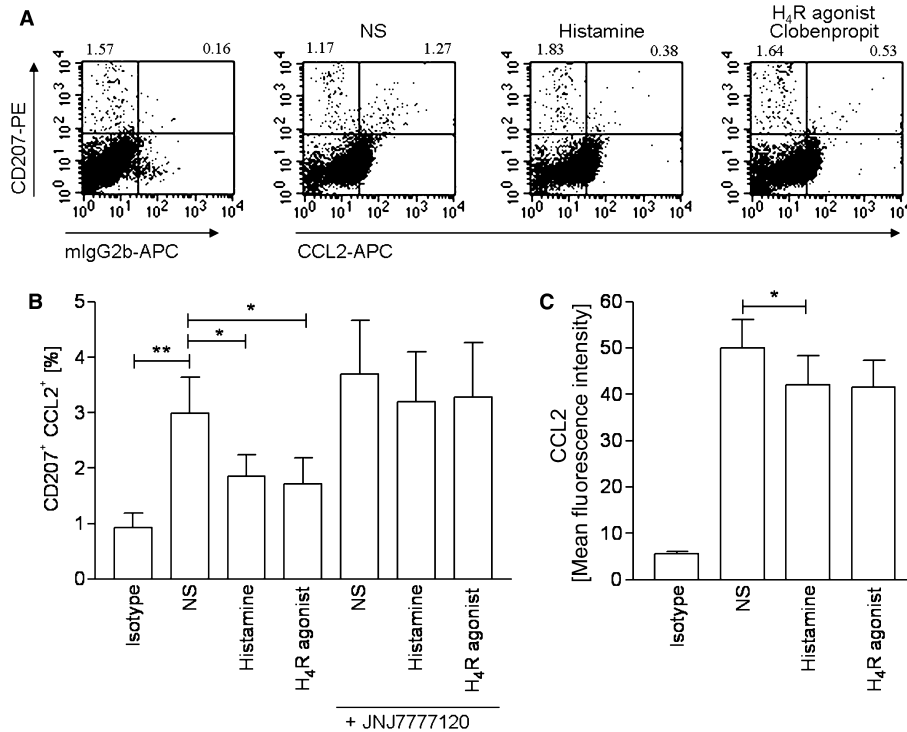


Figure 4 Downregulation of CCL2 in human Langerhans cells (LC) after H₄R stimulation. Intracellular CCL2 is downregulated in LC in epidermal cell suspensions after 48 h of incubation with 10 μ M histamine or the H₄R agonist clobenpropit, percentages of CCL2 negative and positive cells are stated above the dot plots (A). Prestimulation with the H₄R antagonist JNJ777120 blocks the

downregulation of CCL2 (B, mean and SEM of seven independent experiments are depicted). Also, in monocyte-derived LC, a decrease in CCL2 production was detected (C, mean and SEM of four independent experiments are depicted). * $P < 0.05$, ** $P < 0.005$.

because it is predominantly found in the central nervous system (3).

We measured the expression of the H₄R on human MoLC, which were *in vitro* generated from monocytes and on primary *ex vivo* LC isolated from murine and human skin samples (naturally occurring LC). We demonstrate for the first time that murine as well as human LC express the H₄R on the mRNA and protein level, as shown by real-time PCR, flow cytometry and immunofluorescence staining of the skin. Immunomodulatory effects of histamine mediated via the H₄R have already been described; in particular, the induction of migration of a variety of immunologically relevant cell types has been shown, such as mast cells (6), MoDC (7), eosinophils (8) and regulatory T cells (16). Because of these observations and the finding that the migration of murine skin DC can as well be induced by H₄R agonists (15), we were interested in studying the migration of LC in response to histamine. We could show that H₄R stimulation increases the migration of human LC *ex vivo* and of murine LC *in vivo*. Induction of LC migration via the H₄R may be an explana-

tion for the finding that the epidermis overlying lesions of urticaria pigmentosa – a type of mastocytosis with accumulation of mast cells in the dermis – contains a reduced number of LC (17). Because mast cells in the dermis can release histamine upon various stimulations, this might induce the migration of LC from the epidermis resulting in decreased LC numbers in the epidermis.

LC are not only important in the priming of T cell responses, in addition they secrete chemokines which are responsible for recruitment and functional polarization of other cell types involved in inflammatory processes. An example is the chemokine CCL2, which we investigated in this study. CCL2 leads to the recruitment of macrophages, monocytes and T cells and has been associated with Th2 responses (18). As described previously for monocytes and IDEC (9, 10), we also observed a downregulation of CCL2 production in LC mediated via H₄R stimulation. It is tempting to speculate that activation of the H₄R limits the cell influx of inflammatory cells in lesions of allergic skin diseases and contributes to the shift from a Th2

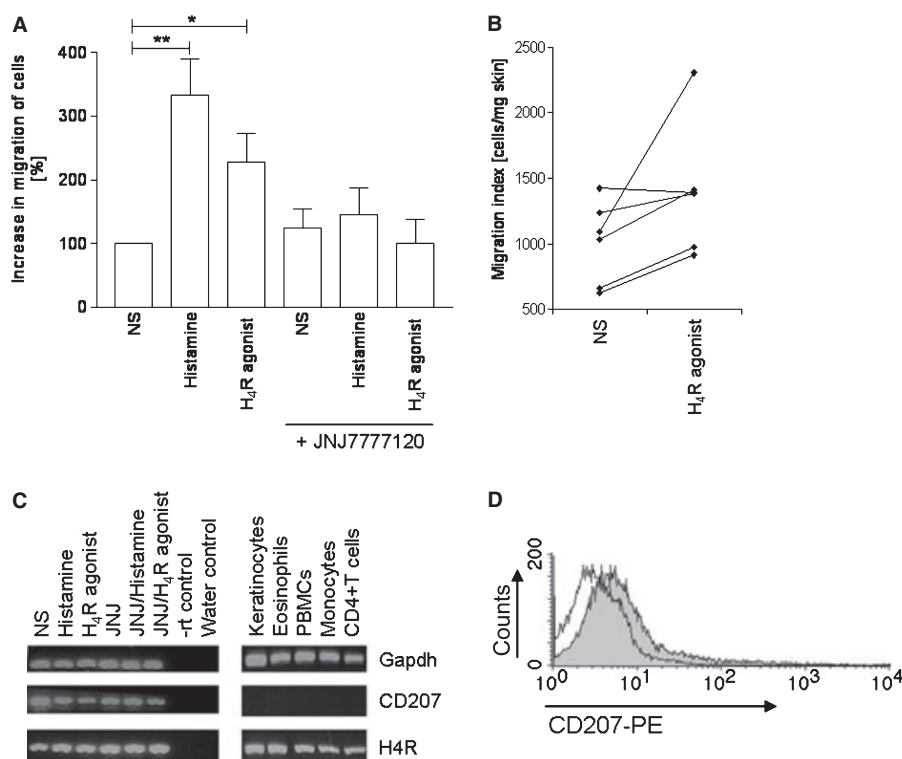


Figure 5 H₄R agonists induce emigration of human Langerhans cells (LC) from the epidermis *ex vivo*. Upon 48 h of incubation of epidermal sheets with histamine or H₄R agonist 4-methylhistamine (10 μ M), LC emigrate from epidermis derived from patients with bullous pemphigoid. This was blocked by the H₄R antagonist JNJ7777120 (A, Mean and SEM of seven independent experiments, normalized to nonstimulated control, 100% equals 1200 LC per mg epidermis). H₄R agonist-induced LC migration

was also observed in epidermis derived from healthy skin (B, $n = 6$, P -value = 0.063). Real-time PCR showing that the migrated cells express CD207 and H₄R mRNA, while control cells do not express CD207, GAPDH was used as reference gene (C, one experiment of three). Flow cytometric evaluation of CD207 expression (D, CD207: grey, isotype: white, one experiment out of three is shown). * $P < 0.05$, ** $P < 0.005$.

to a Th1 milieu as observed during the transition from acute to chronic eczematous skin lesions (19).

Another interesting observation is that CCL2 among other chemokines can activate and thereby degranulate mast cells and basophil granulocytes in an immunoglobulin E (IgE)-independent manner. This mechanism of activation is of particular importance in late-phase reactions. In the murine system, CCL2-dependent activation of mast cells and basophils was shown (20), while in human cells, induction of degranulation and histamine release by CCL2 was only observed in basophils (21). These findings may point at a feedback mechanism, in which the downregulation of CCL2 by H₄R stimulation might be inhibiting the late-phase release of additional histamine from mast cells and basophils. Moreover, in response to the lack of CCL2, also other chemokines such as CCL5 and CCL3 are downregulated (18), which can also function as histamine-releasing factors. Because the decreased production of CCL2 is not only seen in LC but also was

described for monocytes and IDEC (9, 10), this might be a general mechanism of dampening an immune response.

In conclusion, this study clearly shows that histamine, and in particular its action via the H₄R, plays an important role in modulating the function of LC. On one hand, increased migration of LC from the epidermis was observed, probably resulting in faster transport of encountered antigens to the local lymph nodes. On the other hand, we found downregulation of the production of CCL2 presumably resulting in decreased recruitment of macrophages and T cells and impaired IgE-independent release of histamine. These findings indicate that histamine influences the local immune response by two different mechanisms: one ensuring fast presentation of antigens and one preventing an overwhelming immune response. These findings underline the relevance of the H₄R in allergic skin diseases and encourage further exploration of the H₄R as a therapeutic target in allergic diseases.

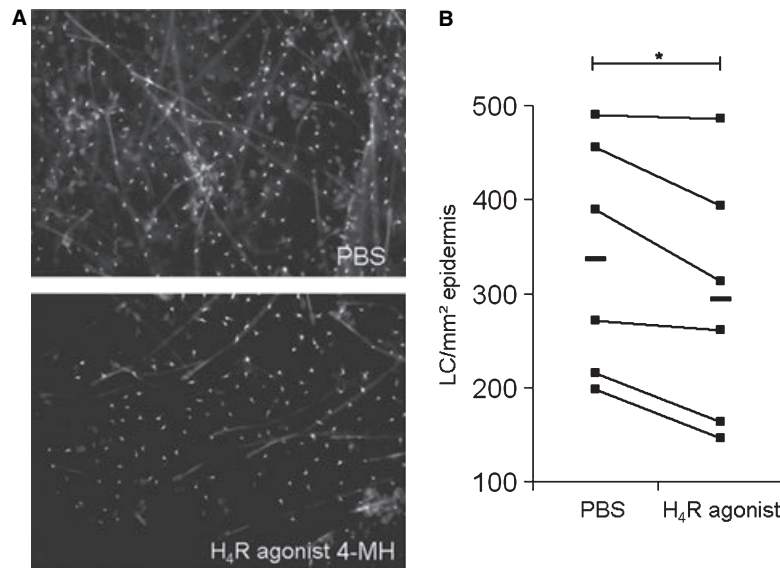


Figure 6 Intradermal injection of a H₄R agonist induces emigration of murine Langerhans cell (LC) from skin *in vivo*. After intradermal injection of 10 μ M 4-methylhistamine (4MH), less MHC-II-positive cells are detected in the epidermis when compared to PBS

control. One representative experiment is shown (A), six independent experiments are summarized in (B) (Data points represent mean numbers of LC/mm² of 16 randomly chosen areas; the line represents the mean of six experiments). **P* < 0.05.

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Conflict of interest

The authors declare no conflict of interest.

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